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Water absorption capability of chickpea in relation to nematode infection

(chickpea/water absorption/parasitic nematodes)

SARTAJ A. TIYAGI, MEHAR BANO, SUHAIL ANVER AND M. MASHKOOR ALAM
Department of Botany, Aligarh Muslim University, Aligarh–202 001

ABSTRACT Water absorption by chickpea roots was retarded due to infection of various nematodes. The retardation in water absorption was found directly correlated to pathogenicity and the damage caused by them.

During a survey of chickpea (*Cicer arietinum* L.) fields in Aligarh, a western district of Uttar Pradesh, it was observed that the unthrifty plants harboured large poulations of the most common nematode species, *viz.*, the root-knot nematode, *Meloidogyne incognita* (Kofoid and White) Chitwood, the reniform nematode, *Rotylenchulus reniformis* Linford and Oliveira and the stunt nematode, *Tylenchorhynchus brassicae* Siddiqi. No report regarding water absorption capability of the nematode infected chickpea roots in relation to plant growth is, however, available. These aspects were, therefore, investigated with these very important species of plant parasitic nematodes having different modes of feeding.

Three-week-old plants of chickpea cv. H-208 growing singly in pots containing 500 g autoclaved soil were inoculated with 1000 specimens of different test species of nematodes as per schedule given in Table 1. Final observations were made 3 months after inoculation. The 'root-knot index' was rated on 0-4 scale. Reproduction factor of the nematode species was calculated by dividing the final population with the initial population ($R=Pf/Pi$). The amount of water absorbed by roots within 24 h was determined by the method described by Alam *et al.*[1]

TABLE 1

Effect of plant parasitic nematodes on the growth and water absorption capability of chickpea cv. H 208.

Nematodes	Plant weight (g) Shoot	Root	Total	$R=\frac{Pf}{Pi}$	Root-knot Index	Water absorbed per Plant (g)
Check	22.60	1.35	23.95	—	—	14.00
Meloidogyne incognita	9.20	0.90	10.10	4.68	3.00	6.00
Rotylenchulus reniformis	8.26	1.13	9.39	5.27	—	8.00
Tylencho-rhynchus brassicae	12.50	2.50	15.00	2.83	—	12.00
C. D. (P=0.05)			2.391			1.194
C. D. (P=0.01)			3.538			1.767

Each value is an average of three replicates.
R = Reproduction factor, Pf = Final population, Pi = Initial population (1000/Plant).

Table 1 shows that all the three species of nematodes tested, significantly inhibited plant growth, which was maximum in plants infected with *R. reniformis* (plant weight=9.39 g) followed by *M. incognita* (10.10 g) and *T. brassicae* (15.00 g) as compared to un-inoculated controls with average plant weight of 23.95 g. The damage to plant growth was found directly correlated with the reproduction factor (multiplication rate) of the nematodes. The many fold increase in the population of nematodes confirms that chickpea cv. H-208 is a good host of the test nematodes. Root-knot nematode also caused gall formation in roots (root-knot index = 3.00). Significant inhibition in water absorption was observed in the infected roots and maximum inhibition was noted in plants infected with *M. incognita* followed by *R. reniformis* and *T. brassicae*, respectively. This is understandable because *M. incognita* is an endoparasite and is known to damage, deform, disrupt and block the conducting tissues of roots by hyperplastic and hypertrophic growth of cells[2]. *R. reniformis*, a semi-endoparasite, causes comparatively less aberrations of the internal tissues[2], while *T. brassicae*, a surface feeder or an ectoparasite, has still less damaging effect[3]. The nematode infection inhibited the growth and the total surface area of roots (Table 1). This may be one reason for poor absorption of water by roots of the nematode infected plants, and the other may be reduced transpiration pull on account of decreased shoot weight/leaf surface area.

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Biochemical changes in the roots of *Triticale* infected with *Puccinia recondita*

(biochemical changes/roots/normal and diseased *Triticale*)

SHABBIR ASHRAF, DHIRENDRA PRAKASH AND IRSHAD MAHMOOD

Section of Plant Pathology and Nematology, Department of Botany, Aligarh Muslim University, Aligarh-202 001

ABSTRACT The total free amino acids, ortho-dihydroxy phenols and phenols were isolated from the root extracts of *Triticale hexaploide* Lart. infected with *Puccinia recondita* Rob. ex Desm. The concentration of free amino acids and phenolics was more in diseased plants than normal ones

It has been known that the physiology and biochemistry of plants are altered due to infection. The physiology of roots in relation to pathogenesis has been studied by many workers. MacRae and Castro[1] studied the root exudates of rice plants in relation to 'Akagare' disease. Rohringer and Sambroski[2] noticed an overall increase in amino acids in rust infected wheat plants. The present paper deals with the biochemical changes in the roots of *Triticale hexaploide* Lart. infected with *Puccinia recondita* Rob. ex Desm.

Seeds of *T. hexaploide* variety DTS-481 were obtained from N.S.C., I.A.R.I. Campus, New Delhi. The seeds were sown at the University Botanical Garden (Fort) and the mature plants (normal as well as those infected with rust, *Puccinia recondita*) were uprooted and brought to laboratory in sterilized polythene bags. Dry roots of normal and diseased plants were powdered separately in an electric grinder and sieved through 60 mesh pore size sieve. Phenol was extracted by the method of Biehn *et al.*[3] by using Folin Cio-calteau reagent. For estimation of ortho-dihydroxy phenol, the method of Johnson and Schaal[4] was followed. Total free amino acids were estimated by the method of Moore and Stein[5], using modified Ninhydrin reagent. Chromatographic separation of individual amino acids was done following the method of Block[6].

Concentration of free amino acids in the roots of diseased plants was 0.22 mg, that of phenol 0.57 mg and that of ortho-dihydroxy phenol 0.435 mg. In the roots of normal plants the concentration was 0.170 mg, 0.285 mg and 0.200 mg, respectively. Amino acids L-cystein, taurine, DL-leucine, isoleucine, DL-methionine, alanine, DL-threonine and serine were found in roots of diseased Triticale plants while L-asparagine, DL-valine, L-glutamic acid and DL-methionine were recorded in those of normal plants. The increase of free amino acids in the roots of infected plants, may be attributed to the altered physiological activities. Most of the amino acids at concentrations normally present in plants are nontoxic to most of the disease causing organisms and may not account for the resistance. However, under certain conditions amino acids may help the plants to develop resistance. There are reports by Tomiyama[7]and Schroth and Hildebrand[8] that certain

amino acids like phenylalanine help in accumulation of phenolic compounds in plants as a result of parasite infection. They also form complexes with phenolic compounds such as amino acid-chlorogenic acid complex which are directly responsible for resistance. The increase in amino acid concentration is probably due to polymerization or splitting by the parasite. It can be concluded that the pathogen brings about considerable changes in the physiology of host. It is also responsible for quantitative and qualitative changes in root extracts.

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Antifungal activity of some 6-arylpyrido [2', 3' : 4, 5] pyrimido [1, 6-a]-benzimidazoles

(fungicide/benzimidazole/*Drechslera spicifer*/*Fusarium solani*)

V. KRISHNA REDDY, S. M. REDDY, K. VIJAYENDER REDDY,* K. MOGILAIAH* AND B. SREENIVASULU*

Department of Botany, Kakatiya University, Warangal-506 009

**Department of Chemistry, Kakatiya University, Warangal*-506 009

ABSTRACT Antifungal activity of ten 6-arylpyrido [2',3' : 4,5]-pyrimido [1,6-a]-benzimidazoles was assayed against *Drechslera spicifer* and *Fusarium solani*. Compound 6-m-nitrophenylpyrido-[2',3' :4,5] pyrimido [1,6-a] benzimidazole was found to be highly active against *D. spicifer*, while compound 6-p-nitrophenylpyrido-[2',3' : 4,5] pyrimido [1,6-a] benzimidazole was active against both the fungi. However, it caused total spore germination inhibition of *Fusarium solani* only at 840 μg/ml concentration. 6-p-Methoxyphenyl compound was found to lack fungicidal activity against both the fungi under investigation.

Though a number of benzimidazole derivatives are in extensive use as fungicide[1,2] and bactericides[3,4], pyridopyrimidines, which are reported to be bactericidal[5], have not yet been exploited as fungicides. Recently Vijayender Reddy *et al.*[6], have reported the synthesis of 6-arylpyrido [2', 3' ; 4, 5] pyrimido-[1,6-a] benzimidazoles which are having both the moeities. Hence, in the present investigations, the fungicidal activity of some derivatives of 6-arylpyrido-[2',3' : 4,5] pyrimido-[1,6-a] benzimidazoles was evaluated and discussed in this communication.

Fungicidal activity or some substituents of 6-arylpyrido-[2',3' : 4,5] pyrimido [1,6-a] benzimidazoles (as detailed in Table 1) against *Drechslera spicifer* and *Fusarium solani*, seed-borne fungi of maize and pearl millet respectively, was evaluated by glass slide-humid chamber technique[7]. Different concentrations of the test compounds [360,600 and 840 μg/ml) were prepared by dissolving 30 mg of compounds in 10 ml of acetone and diluted subsequently by adding distilled water. The solvent treated in a similar manner without the compound served as control. The spore suspension of test fungi was prepared from 7 days old fungus and the spore concentration was adjusted so as to appear 20-30 spores per microscopic field (L.P.). The details of spore germination was recorded at the end of 12 h, as by which time most of the spores germinated and accomplished considerable growth. At least 250-300 spores in 10 randomly selected microscopic fields (L.P.) were scored to calculate the percentage of spore germination inhibition by using the formula ;

$$\text{Percentage of inhibition} = 100 - \frac{\%\text{ of germination in treatment}}{\%\text{ of germination in control}} \times 100$$

Table 1 reveals that different substituents or 6-arylpyrido-[2',3' : 4,5] pyrimido [1,6-a] benzimidazoles exhibited fungicidal activity which, however, varied both with the test compound and fungus. Compound 7 was highly toxic which caused total spore germination inhibition of *D. spicifer* even at 360 μg/ml concentration. However, it was responsible for partial spore germination inhibition of

TABLE 1

Antifungal activity data of 6-arylpyrido [2′, 3′ : 4, 5] pyrimido [1,6-a]-benzimidazoles

Sl. No.	Pyrido [2′,3′ : 4,5] pyrimido [1,6-a] benzimidazole	Concentration (in μg/ml)	Percentage of spore germination inhibition	
			D. spicifer	*F.solani*
1.	6-phenyl	360	—	—
		600	21.14	14.54
		840	71.00	68.34
2.	6-p-methylphenyl	360	12.73	—
		600	63.95	21.14
		840	100.00	84.19
3.	6-p-methoxyphenyl	360	—	—
		600	—	—
		840	—	—
4.	6-p-chlorophenyl	360	—	15.51
		600	21.25	56.77
		840	69.20	76.15
5.	6-p-bromophenyl	360	17.20	—
		600	77.10	47 50
		840	100.00	80.00
6.	6-p-nitrophenyl	360	38.50	18.83
		600	100.00	50.00
		840	100.00	100 00
7.	6-m-nitrophenyl	360	100.00	13.30
		600	100 00	21.28
		840	100 00	85.86
8.	10-nitro-6-p-chloro-phenyl	360	—	—
		600	23.52	20.67
		840	100.00	88.00
9.	6-α-thienyl	360	47.52	—
		600	100.00	51.92
		840	100 00	84.19
10.	9-nitro-6-α-thienyl	360	13.53	—
		600	100.00	48.59
		840	100.00	93.37

– Indicates no acitivity

F. solani. Compound 6 comes next in the order of toxicity which was responsible for total germination inhibition of *D. spicifer* and *F. solani* at 600 μg/ml and 840 μg/ml concentrations respectively. Compound 3 was found to lack fungicidal activity. Compound 1 and 4 exhibited low fungicidal activity and required more than 600 μg/ml concentration for more than 50% of spore germination inhibition. Rest of the compounds were intermediate in their fungitoxicity. In general, *F. solani* was comparatively more resistant to these compounds than *D. spicifer*. From the present investigations it can be concluded that nitro group both at para and meta position is active in fungitoxicity. The lack of fungi toxicity of compound 3 may be attributed to methoxyl group at para position. Comparatively low toxicity of compound 8 than compound 7 may be attributed to the addition of chloro group.

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Water quality and corrosion

(water quality/corrosion)

VIRENDRA SINGH, MRIDULA SINGHVI AND B. C. L. AGARWAL

Department of Chemistry, Malviya Regional Engineering College, Jaipur

ABSTRACT Tubewell water of Malviya Nagar, Jaipur was found to be initially more corrosive to commercial aluminium samples than deionised water, 1% and 3% NaCl solutions. Probable mechanism explaining the higher corrosion rate in tubewell water is suggested.

The formation of carbonate scale in Malviya Nagar, Jaipur, has been explained earlier[1] on the basis of water characteristics of the area. Recent studies on corrosion of commercial samples of aluminium showed that the metal corrodes faster in tubewell water of this area than in deionised water, in 1% and 3% NaCl solutions in deionised water. These investigations need attention as articles of daily use such as utensils, radiators used for automotive cooling systems and parts of equipments used in industries are mostly made of aluminium and its alloys.

A comparative study of corrosion rates of aluminium in tubewell water, deionised water, 1% and 3% NaCl solutions in deionised water has been made here and the probable mechanism is discussed to throw more light on the subject.

Aluminium samples (commercial grade) of $3 \times 2 \times .05$cm were taken and cleaned by standard methods[2]. Weight loss in these samples were determined in different media using a single pan balance of Owa Labor make. Samples were treated with fresh solutions each time and the corrosion rate was calculated in all the sets in mpy[2].

The corrosion rate in tubewell water is initially higher than in other corroding media which decreases after reaching a maximum in about 5 days. Corrosion in 1% NaCl solution is similar but slower. Corrosion behaviour in deionised water and in 3% NaCl solution is different. The rate of corrosion shows an alternate increase and decrease with time. However, the corrosion rate shows a large decrease after 24 h with 3% NaCl solution.

TABLE 1

Changes in pH of corroding medium

Medium	pH	
	Initial	After 3 days
Tubewell water	7.98	8.78
Tubewell water + Al sample	8.78	8.53
Deionised water + Al sample	7.70	7.52
1% NaCl solution + Al sample	7.62	7.50
3% NaCl solution + Al sample	7.67	7.56

The modern electrochemical theory interprets the surface of a corroding metal as a single electrode on which coupled reactions of spontaneous dissolution take place simultaneously and statically independently[3]. At the first stage there appear intermediary chemisorbed complexes consisting of particles of the solution and surface atoms of the metal. At subsequent stages the complexes are ionised, pass into the solution and then dissociate. In case of tubewell water whose characteristics were published earlier[1], it appears that the initial higher rates are

due to higher pH of the medium which increases from 7.98 to 8.78 in about 24h due to sodium glasses. Such increase in pH has been observed by Singh[4] during filtration of Kohlschutter's silver sol through glass wool. It is also observed that the pH of all the solutions with metals showed a decrease in pH (Table 1). These observations lead us to believe that the dissolution reaction with tubewell water is

$$Al + H_2O + OH^- \rightarrow AlO_2^- + \frac{3}{2} H_2$$

which occurs as a result of adsorption of water and hydroxyl ions on the metal surface. Such corrosion reactions are reported in cathodic dissolution of aluminium[5]. The above reaction also consumes hydroxyl ions resulting in decrease of pH.

The decrease in corrosion rate after reaching a maximum in about 5 days in the tubewell water may be the result of decrease in surface area due to the deposition of suspended particles of tubewell water as actually observed.

The low hydroxyl ion concentration is responsible for comparative slow rate of corrosion observed initially with deionised water. The alternate increase and decrease in corrosion rate seems to be on account of variations in the rate of diffusion of hydroxyl ions through the protective layer of water molecules. A large decrease in corrosion rate after 24 h with 3% NaCl solution is due to decrease in corrosion rate with time. Our experiments with stagnant 3% NaCl solutions confirm this view. The decrease in corrosion rate in stagnant solution could be explained by the slow rate of dissociation of the complex in the following mechanism suggested by Kolotyrking[6].

$$Al + 3\,Cl^- \rightleftharpoons AlCl_3 + 3e^-$$

$$AlCl_3 + nH_2O \rightleftharpoons AlCl_3\,(H_2O)_n$$

$$AlCl_3(H_2O)_n \rightleftharpoons Al^{+3} + 3Cl^- + nH_2O$$

The corrosion rate is low with 1% NaCl solution due to slow rate of diffusion of chloride ions in the first step in the above mechanism.

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Stereochemical investigation of furfuryl alcohol maleic anhydride adduct by NMR spectroscopy

(NMR/stereochemical study/furfuryl alcohol/maleic anhydride)

MAMTA

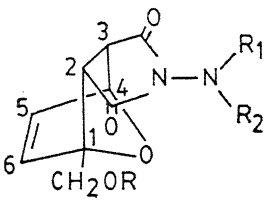
Department of Chemistry, University of Allahabad, Allahabad

ABSTRACT Non-planar stable conformation and hindered rotation about N-N′ bond in N′-diacylaminoimide derivatives of furfuryl alcohol-maleic anhydride adduct have been studied by NMR spectroscopy. The signal duplicity in the NMR spectrum for the two N′-acetyl groups in N′-diacetylaminoimide derivative of furfuryl alcohol-maleic anhydride adduct arises due to asymmetric cage moiety about the succinimidyl plane, suggesting for the *exo* configuration.

The existence of non-planar ground state and restricted rotation about N-N bond in N-(diacylamino) imide systems is a well documented phenomenon[1-4]. The two acetyl groups attached to exocyclic nitrogen experience different magnetic environment in presence of asymmetric cage moiety. The experience of different magnetic environment of N′-(diacetylamino) imide system in NMR spectrum (lying nearer to the cage moiety) probes into the magnetic effect of the dienyl part of the adduct, whereby the configuration of the adduct could be determined[1-6]. Here a technique has been utilized for the configurational assignment of the Diels-Alder adduct of furfuryl alcohol-maleic anhydride[7].

7-oxabicycle (2.2.1)-*5-heptene-1-hydroxymethyl-2, 3-exo dicarboxylic anhydride* : It was obtained from the method used by Hodgson and Devies[7] for the preparation of 4-hydroxy-3, 6-endoxo- Δ^4 tetrahydrophthalic anhydride.

N - amino - 7 - oxabicycle (2.2.1)-*5-heptene-1-hydroxymethyl - 2, 3-exo-dicarboximide* (I) : This compound (Fig. 1) was prepared by stirring a mixture of *exo* furfuryl alcohol-maleic anhydride adduct (1 mole) and hydrazine hydrate (1 mole) in ethanol at room temperature. The stirring continued for an hour to complete the reaction and then the separated mass was filtered, dried and recrystallised with ethanol, m.p. 155°.



(I)	R = H R_1 = H R_2 = H	(IV)	R = $COCH_3$ R_1 = $COCH_3$ R_2 = $COCH_3$
(II)	R = H R_1 = COPh R_2 = H	(V)	R = $COCH_3$ R_1 = COPh R_2 = H
(III)	R = H R_1 = C_6H_5 R_2 = H	(VI)	R = $COCH_3$ R_1 = C_6H_5 R_2 = H

Fig. 1. Derivatives of furfuryl alcohol

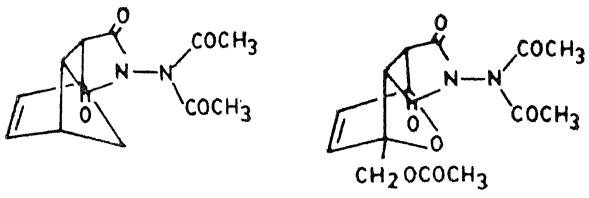
N′-(monobenzoylamino) imide derivatives (II *and* V) : These compounds (Fig. 1) were prepared by

stirring the equimolar amount of the adducts and benzoylhydrazine in ethanol at room temperature for 2 h. After removing the solvent the products were recrystallised from ethanol, m.p. were 177° and 167° respectively.

N'-anilinoimide derivatives (III & VI) : These compounds (Fig. 1) were prepared by adding phenylhydrazine dropwise, while stirring to an equimolar amount of the corresponding adducts in ethanol at room temperature. The stirring was continued for an hour to complete the reaction. Thus, the solid obtained was filtered and recrystallised from ethanol.

N'-(diacetylamino)-7-oxabicyclo (2.2.1)-5 *heptene-1-acetyl methyl-2, 3-exo dicarboximide* (IV) : This compound (Fig. 1) was obtained by heating the N-aminoimide (I) with an excess of acetic anhydride on water bath for 1 h and was then kept for 24 h at room temperature. The separated crystals were filtered and recrystallised from ethanol, m.p. 144–145°.

The NMR spectrum of N', N'-diacetyl derivative (IV) of furfuryl alcohol–maleic anhydride adduct (I) shows a pair of singlets at δ 2.06 (3H) and δ 2.34 (3H) for the two N'-acetyl groups ($\triangle\nu$=26.5 Hz), an A B quartet of 2H intensity at 3.11 for C–2 and C-3 methine protons and a singlet of 1H intensity for C-4 methine proton at δ 5.33 along with the other normal proton resonances. Duplication in the acetyl resonance indicates the presence of a non-planar conformation and restricted rotation about the N–N bond. The spectral pattern of this compound is very much similar to N'-diacetyl derivative of N-amino (2.2.1) bicyclo-5-heptene-2, 3-exo dicarboximide (1) (shown in Fig. 2) except a change in the internal c emical shift of the N'-acetyl protons ($\triangle\nu$=16.5 Hz) [8,9]. As a result of non-planar conformation about the N-N bond in the present system, one of the acetyl group lies above and the other below the plane of the succinimidyl ring, *i.e.* one acetyl group, *syn* to the oxygen bridge, falls in its deshielding zone, resonates at downfield. The other acetyl group lying above the plane of succinimidyl ring, shows the normal resonance as it is not much effected by the cage moiety. Hence the two N'-acetyl groups experience two different magnetic environments due to magnetic asymmetry of the cage moiety about the succinimidyl plane and give rise to two different signals in the NMR spectrum. On this consideration the compound (IV) could be given an *exo* configuration (2) (shown in Fig. 2).



(1) (2)

Fig. 2. (1) N'-diacetyl derivative ot N-amino (2.2.1) bicyclo-5-heptene-2,3-exo dicarboximide, (2) N'-(diacetyl amino)-7-oxabicyclo (2.2.1)-5-heptene-1-acetyl methyl-2,3-exo dicarboximide

N'-aroyl aminoimide and N'-phenyl-N'-aminoimide systems are other versatile probes for the direct determination of the configuration of the adduct.

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Kinetics of oxidation of acetoin by bromamine-T in feebly alkaline medium

(kinetics/oxidation/acetoin/bromamine-T/alkaline medium)

KUNJ BEHARI AND MADHU GAUTAM

Chemical Laboratories, University of Allahabad, Allahabad-211 002

ABSTRACT The oxidation of first compound of α-hydroxy ketone series *i.e.* acetoin by using alkaline sodium N-bromo-p-toluenesulpronamide or bromamine-T (BAT) presents the initiative work of bromamine-T-α-hydroxyketone system confirming that enolisation is a rate determining step.

Kinetics of oxidation of acetoin has been studied by many workers in acidic[1] and alkaline medium[2]. The kinetic and mechanistic studies of different compounds employing bromamine-T[3-9] have also been made by different workers but no information is available about the oxidation of this series of compounds involving bromamine-T in feebly alkaline medium.

Bromamine-T and buffer solutions of different pH were prepared as reported elsewhere[10,11] and strength of aqueous solution of acetoin was checked by hydroxylamine hydrochloride-pyridine procedure[12]. Isolation condition *i.e.* [acetoin] ⩾ [BAT] has been maintained throughout the work. The study has been made in 25% buffer solution. Due to interference of liberated iodine in direct titration of bromamine-T, kinetics was followed by estimating the oxidant ascorbimetrically, using chloramine-T as a titrant and KI-starch as indicator.

Reaction follows zero-order dependence on bromamine-T, showing thereby that oxidation proceeds *via* enolisation.

In aqueous alkaline solution, bromamine-T exists[13] as follows :

$$\underset{\text{(BAT)}}{CH_3C_6H_4SO_2NBrNa} + H_2O \rightleftharpoons \underset{\text{(BATH)}}{CH_3C_6H_4SO_2NHBr} + NaOH \quad (1)$$

$$CH_3C_6H_4SO_2NHBr + NaOH \rightleftharpoons \underset{\text{(TSA)}}{CH_3C_6H_4SO_2NH_2} + NaOBr \quad (2)$$

Therefore, in alkaline medium, BAT exists as BAT itself, protonated BAT (p-toluenesulphobromamide or BATH) and sodium hypobromite and only one of these three will be involved in oxidation of acetoin.

Highlighting the evidence[3] "formation of HOBr in strongly alkaline solutions", Ruff and Kucsman[5] have proposed that [BATH] ⩽ [HOBr] if pH > 12, but in the present case study has been made in feebly alkaline pH (8.1 - 8.9) and, therefore. HOBr cannot be considered as a main reactive species.

Independence of reaction rate on increasing dielectr c constant gives the same information, since alcohols are oxidised at very convenient rate with bromine in alkaline medium where HOBr is responsible for the oxidation[14]. Further, the observation that ionic strength has no effect on the reaction rate presents a valuable support that atleast one neutral molecule is involved.

The value of different thermodynamic parameters have been calculated by studying the reaction at

TABLE 1

Effect of temperature on rate of reaction

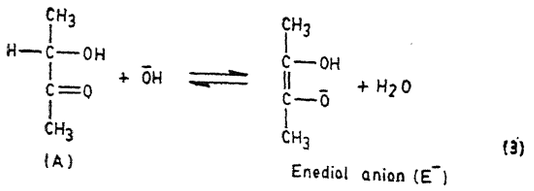
[BAT] = 2.0 × 10^{-3}M pH = 8.5

[acetoin] × 10^3M	k_s × 10^6 (M min^{-1})				
	25°	30°	35°	40°	45°
100.00	57.00	108.60	240.00	–	–
50.00	35.38	67.12	124.00	220.00	–
20.00	18.28	29.90	47.34	81.00	134.00
12.50	10.90	18.76	30.16	46.66	70.60
10.00	9.30	15.12	24.00	37.40	56.00

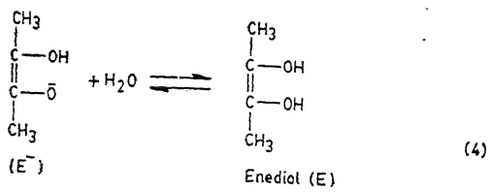
five temperatures (Table 1). The low value of thermodynamic parameters ($\triangle E^{\neq} = 20.00$ kcal/mole, $\triangle S^{\neq} = -129.07$ e. u. and $\triangle F^{\neq} = 18.47$ kcal/mole at 25°) support the view that similarly charged ions are not involved.

It is observed that as pH is decreased, reaction is remarkably slowed down (near pH=7) and at pH=4, the reaction does not proceed at all, though acetoin exists in enolic form, meaning thereby that protonated BAT cannot be considered as a reactive species (because there is more probability of formation of BATH in lower pH=4).

On the other hand, it is well known that in alkaline medium, α–hydroxy aldehydes and ketones form their corresponding enol[15] or enol anions[16,17]. Now the direct proportionality of reaction rate with respect to hydroxyl ion suggests that the reactive species is not the enediol but enediol anion[2]. Formation of enediol and enediol anion may be shown as follows :



(3)



(4)

Addition of deaerated reaction mixture to aqueous acrylamide did not initiate polymerisation showing thereby that free radicals are not formed, confirming that instead of homolytic mechanism, heterolytic mechanism is involved. Thus on the basis of above observed facts, two tentative schemes are proposed :

Scheme - 1

$$A + \bar{O}H \underset{k_{-1}}{\overset{k_1 \text{ (slow)}}{\rightleftharpoons}} E^- + H_2O \qquad (3)$$

$$E^- + H_2O \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} E + \bar{O}H \qquad (4)$$

$$E + BAT \xrightarrow{k_3 \text{ (fast)}} \text{Products} \qquad (5)$$

Considering the above steps involved during oxidation, the rate derived is as follows :

$$\frac{-d[BAT]}{dt} = \frac{2 k_1 k_2 k_3 [A][\bar{O}H][BAT]}{k_{-1}k_{-2}[\bar{O}H] + (k_{-1} + k_2)\, k_3\, [BAT]} \qquad (6)$$

The rate law (6) shows first order dependence in $\bar{O}H$, at its lower concentration and zero order dependence at higher concentration which is contradictory to our experimental findings.

Scheme - 2

$$A + \bar{O}H \underset{k_{-1}}{\overset{k_1 \text{ (slow)}}{\rightleftharpoons}} E^- + H_2O \qquad (3)$$

$$E^- + BAT \xrightarrow{k_2 \text{ (fast)}} \text{Products} \qquad (7)$$

Similarly, the rate law can be written as :

$$\frac{-d[BAT]}{dt} = 2 k_1 [E][\bar{O}H] \qquad (8)$$

The factor 2 is introduced because one molecule of acetoin requires two molecules of BAT.

Here k_1 is forward velocity constant and is expressed as rate of oxidation of acetoin, which comes out to be 1.35 m^{-1} s^{-1} at 35°.

TABLE 2

Effect of [BAT], [acetoin] and [$\bar{O}H^-$] on the rate of reaction

Temp. 35°						
[acetoin] = 12.50 × 10^{-3}M		[BAT] = 2.00 × 10^{-3}M		[acetoin] = 12.50 × 10^{-3}M		
pH = 8.5		pH = 8.5		[BAT] = 2.00 × 10^{-3}M		
[BAT] × 10^{3}M	$k_s \times 10^6$ (M min^{-1})	[acetoin] × 10^{3}M	$k_s \times 10^6$ (M min^{-1})	pH	[$\bar{O}H$] × 10^{5}M	$k_s \times 10^6$ (M min^{-1})
4.00	20.54	100.0	240.00	8.1	1.259	12.98
2.80	20.60	50.00	124.00	8.3	1.995	20.58
2.00	20.58	20.00	47.34	8.5	3.162	30.16
1.20	19.50	12.50	30.16	8.6	3.981	40 00
0.80	19.66	10.00	24.00	8.7	5.012	49.90
		6.67	16.00	8.9	7.943	80.00

The rate law (8) successfully verifies the validity of experimental results (Table 2) *i.e.* reaction is zero order in bromamine-T, first order dependence in hydroxyl ion and acetoin and also accords well with the stoichiometry, no effect of ionic strength (μ = 0.0326 – 0.663M), p – toluene sulphonamide (0–5.0 × 10^{-3}M) and dielectric constant (5%-25% alcohol) are also in good agreement with the values of various thermodynamic parameters. The product formed is acetic acid which is confirmed by paper chromatography.

Thus it is concluded both experimentally and theoretically that it is the enediol anion which is responsible for oxidation and it is BAT itself, which is playing a role of real reactive species in fast step. Involvement of enediol anion with BAT in fast step is also supported by the fact that if an organic compound which has no hydrogen atom *e.g.* formaldehyde or benzaldehyde is subjected to oxidation, no oxidation occurs.

It is important to note that this simple compound of acyloin series is chosen first to test the oxidative behaviour of BAT–α–hydroxyketone system in general and the valuable information behind the present investigation is that only the enolisable carbonyl compounds *i.e.* compounds having hydrogen at α–carbon atom can only be oxidised by this oxidant.

On the other hand, it is well known that acetoin dehydrogenase (enzyme) present in bacteria converts acetoin into diacetyl. So keeping this in mind the oxidation of acetoin (a biologically important compound) was carried out by bromamine–T in feebly alkaline medium and it was found that oxidation product of acetoin is acetic acid and not the diacetyl. Therefore, the aim of the present study is not only to point out the oxidation of acetoin but also to confirm the arrival of products reaching in the reactions step by step. These contradictory results (reaction products are diacetyl and acetic acid in these two cases) present the valuable information that any type of hindrance may shuttle down whole of the phenomena which in turn will affect the physiology of human being in some way thus leaving a scope for biologists and chemists both.

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Coherent edge-imaging properties of partially apodised optical systems using Straubel pupils

(coherent edge imaging/apodised optical systems/Straubel pupils)

D. PARDHA SARADHI, T. VENKAT REDDY*, K. P. RAO** AND P. K. MONDAL**

Department of Physics, Sri Satya Sai Institute of Higher Learning, Prasanthinilayam
**Department of Physics, Kakatiya University, Warangal*-506 009
***Optics Research Group, Department of Physics, Osmania University, Hyderabad*-500 007

ABSTRACT The problem of coherent imagery of edge objects with partial apodisation of the pupil by Straubel class of filters has been discussed. The apodisation is effected over a specified area on the pupil. The width and the position of the apodised ring on the pupil have been varied. A few systems have been identified with negligible edge-ringing with reduced edge-shifting.

Apodisation of a pupil to eliminate or to reduce the edge-ringing phenomenon in the coherent images of straight edges has been studied with considerable interest[1-5]. As a result of these studies, it is also established that the edge-shifting phenomenon is minimum with Airy pupils and it increases with apodisation because of the reduction in the transmitted flux through the pupil. Increased edge-shifting impairs the image definition to a large extent. In order to have a suitable compromise between the reduced edge-ringing and the consequent increased edge-shifting, one should be careful in employing the apodisation technique to coherent edge-imaging. In order to overcome this difficulty, at least partially, we have thought of partial apodisation of the pupil[6] and the present work explores the possibility of employing these techniques for obtaining better edge images that could be reasonably free from edge-ringing.

The Straubel apodisation pupil functions are represented by

$$\begin{aligned} f(x,y) &= [1-(x^2+y^2)]^p \text{ for } [x^2+y^2] \leqslant 1 \\ &= 0 \qquad\qquad \text{for } [x^2+y^2] > 1 \end{aligned} \tag{1}$$

The pupil function $f(x,y)$ for partially apodised systems may be written as

$$\begin{aligned} f(x,y) &= 1 \text{ for } 0 < (x^2+y^2) \leqslant \rho_1^2 \\ &= [1-(x^2+y^2)]^p \text{ for } \rho_1^2 < (x^2+y^2) \leqslant \rho_2^2 \\ &= 1 \qquad\qquad \text{for } \rho_2^2 < (x^2+y^2) \leqslant 1 \\ &= 0 \qquad\qquad \text{otherwise} \end{aligned} \tag{2}$$

An opaque straight edge object can be represented by the amplitude transmission

$$\begin{aligned} A(u,v) &= 0 \text{ for } u < 0 \\ &= 1 \text{ for } u \geqslant 0 \end{aligned} \tag{3}$$

The reduced coordinates (u,v) in the object plane are given by

$$u = \frac{2\pi}{\lambda}(n \sin a)\,\xi$$

$$v = \frac{2\pi}{\lambda}(n \sin a)\,\eta \tag{4}$$

where ξ and η are the cartesian coordinates in the object plane. The object spectrum which is the Fourier transform of (3) is given by

$$a(x,y) = \int_{-\infty}^{\infty}\int A(u,v)\, e^{-2\pi i (ux+vy)}\, du\,dv \tag{5}$$

The modified object spectrum is obtained by multiplying $a\,(x,y)$ with the pupil function *i.e.*

$$a'\,(x\,y) = a\,(x,y)\,f\,(x,y) \qquad (6)$$

The inverse Fourier transform of (6) gives us the complex amplitude distribution in the image plane. Thus,

$$A\,(u,v) = \int_{-\infty}^{\infty}\int a'\,(x,y)\,e^{2\pi i(ux+vy)}\,dxdy \qquad (7)$$

Using eqns. (2-6) in (7) and simplifying

$$A'\,(u',v') = \frac{1}{2} + \frac{1}{\pi}\,[\mathrm{Si}\,(\rho_1 Z) + \mathrm{Si}\,(Z) - \mathrm{Si}\,(\rho_2 Z) + \int_{\rho_1}^{\rho_2} \frac{(1-x^2)^p}{x}\,\mathrm{Sin}\,(Z\,x)\,dx] \qquad (8)$$

For Airy type of pupils $\rho_1 = \rho_2$ and we get

$$A'\,(u',v') = \frac{1}{2} + \frac{\mathrm{Si}\,(Z)}{\pi} \qquad (9)$$

In eqns. (8) and (9) Si(Z) stands for the standard sine integral given by[7]

$$\mathrm{Si}(Z) = \int_0^1 \frac{\sin(Z\,x)}{x}\,dx \qquad (10)$$

where $Z = 2\pi u'$ (11)

For a fully apodised pupil, $\rho_1 = 0$ and $\rho_2 = 1$. We thus get from eqn. (8),

$$A'(u',v') = \frac{1}{2} + \frac{1}{\pi}\int_0^1 \frac{(1-x^2)^p}{x}\,\sin\,(Zx)\,dx \qquad (12)$$

The intensity distribution in the image plane is obtained by taking the squared modulus of (12). The integral occuring in (8) has been evaluated numerically using the 9-point gaussian quadrature on a PDP-11 computer.

In our study of coherent imagery of straight edges, we have used Straubel class of apodisation filters to determine edge-ringing, edge-shifting and edge-spread. The edge-ringing is the difference between the first maximum intensity of the edge fringes and the object intensity level. The distance of the image edge at half intensity value of the object edge from the geometrical edge is a measure of the edge shifting. The distance in the image plane, separating the points of 10% and 90% intensity value of the object edge is measure of the edge-spread. We have selected four configurations (out of 22 configurations)[6] *viz.* 5,9,12 and 17. These are found to have a very low value of negative amplitude response which is best suited for minimising the edge-ringing. The intensity distribution with increasing Z for values of apodisation parameters $P = 1, 2, 3$ and 4 was studied in the case of the

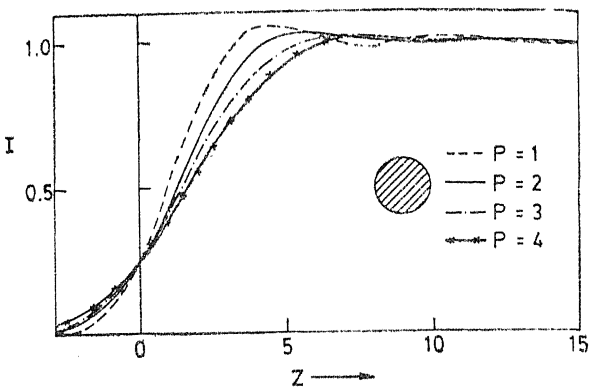

Fig. 1

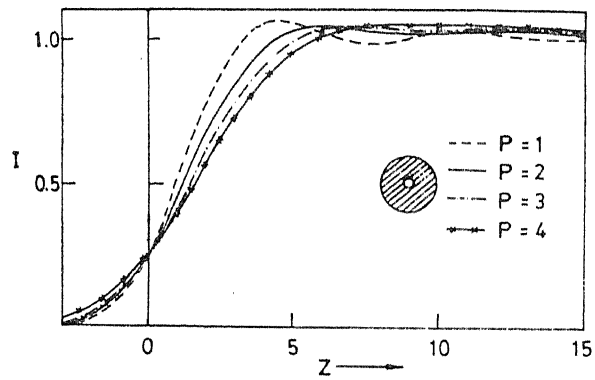

Fig. 2

TABLE 1

Edge-ringing, edge-shifting and edge-spread for different values of P for different configurations

Configuration	Parameter	$P=1$	$P=2$	$P=3$	$P=4$	$P=5$
5	Edge-ringing	–	–	–	–	–
	Edge-shifting	1.40	2.15	2.85	3.55	4.40
	Edge-spread	6.20	14.80	-*	-*	-*
9	Edge-ringing	–	–	–	–	–
	Edge-shifting	1.20	1.85	2.25	2.60	2.85
	Edge-spread	5.15	7.15	8.5	9.00	9.75
12	Edge-ringing	0.09	0.14	0.16	0.17	0.18
	Edge-shifting	1.20	1.40	1.55	1.65	1.65
	Edge spread	4.05	4.85	5.40	5.50	5.50
17	Edge-ringing	0 02	-0.01	-0.04	-0.085	-0.125
	Edge-shifting	1.20	1.60	1.80	2.00	2.05
	Edge-spread	4.35	5.65	6.65	7.70	-*

* Max. intensity is below 0.9
Edge-spread is greater than 15.

above mentioned four configurations. It is found from our studies that for configurations 5 and 9 (Fig. 1 & 2), higher values of P have a smoothing effect on the feet as well as on the edge fringes but the edge-shifting becomes very much pronounced. For values of $P = 3$ and 4, there is practically no edge-ringing in configurations 5 and 9 (Table 1). A compromising value of the apodisation parameter which simultaneously restricts edge-ringing and edge-shifting to a reasonable extent in the case of Straubel pupils can be taken as $P = 4$ for configurations 5 and 9. The values of edge-ringing, edge-shifting and edge-spread for $P = 1, 2, 3, 4$ and 5 for configurations 5, 9, 12 and 17 are shown in Table 1. It is found that for the above four configurations, edge-spread increases with increasing values of P. In between configurations 5 and 9, the configuration 9 seems to be better from the view-point of imaging characteristics.

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Short term electroencephalographic investigation of tritium toxicity in Swiss albino mice

(EEG/tritiated water/mice)

A. L. Bhatia, Amita Saraswat, Rashmi Sisodia and G. L. Heda

Radiation Biology Laboratory, University of Rajasthan, Jaipur

ABSTRACT Effects of a single moderate exposure of HTO injection (20/μCi/g body weight *i.e.* 6.12 rad/day or 0.061 Gy/day, Initial dose rate) on the EEG pattern of adult Swiss albino mice have been studied. There is an altered bioelectrical activity of the cerebral region in the form of increased frequencies and a significant lowering in amplitude at 120th h after HTO-administration.

As a radiation hazard of neurobiological significance tritium (^3H ; physical half life 12.3 Yr)[1] an unstable radionuclide of hydrogen has largely been ignored, though it has been demonstrated that during HTO exposure relatively more tritium is taken up by the brain which is retained for relatively longer duration[2,3]. Some preliminary neuromorphometrical observations on tritium toxicity in developing and adult mice brain showed that exposure to tritiated water results in the loss of brain and body weight, neuronal population and brain width and length[4]. The possible resultant effects of HTO in the electrophysiology cannot be safely ruled out. Present investigation is an attempt to study the short-term effects of tritium on the EEG (electroencephalographic) wave pattern of adult mice.

Adult male Swiss albino mice (nos. 4) were anaesthetized with thiopentone (0.035 mg/g of body weight) and ether. Their skulls were drilled in the parietal region at 2 points, symmetrically, in each half and silver-plated metal electrodes were fixed using dental cement. The animals were given at least 3 to 5 days of recovery time from surgery till a consistent wave pattern without noise was achieved. Control or sham-irradiated were taken, the recordings made initially, prior to tritiated water injection, which corresponds to '0' h in Fig. 1. EEG (electroencephalogram) of these animals was recorded on a 4-channel electroencephalograph (Model EC type MDE 76, with ink writer). The paper drive was run at 2 speeds, 1.5 and 30 mm/sec. Then the animals were injected intraperitoneally with 20 μCi (740 kBq)/g body weight HTO. Tritiated water (HTO) was procured from BARC Trombay, Bombay (specific activity 10 mCi or 370 MBq/ml). The EEG recordings were made at various time intervals ranging from 1 to 120 h, post injection and their frequencies and amplitudes were compared with those of control. For the statistical evaluation of quantitative data, Student's t-test was used[5]. The injected activity of 20 μCi (740 kBq)/g body weight has been estimated to impart the initial dose rate of 6.12 rad or 0.061 Gy/day, calculated by the formula as mentioned by Shapiro[6].

As depicted in Fig. 1, analysis of frequencies show no significant changes in treated mice till 20th h post injection when it becomes significant at 0.01

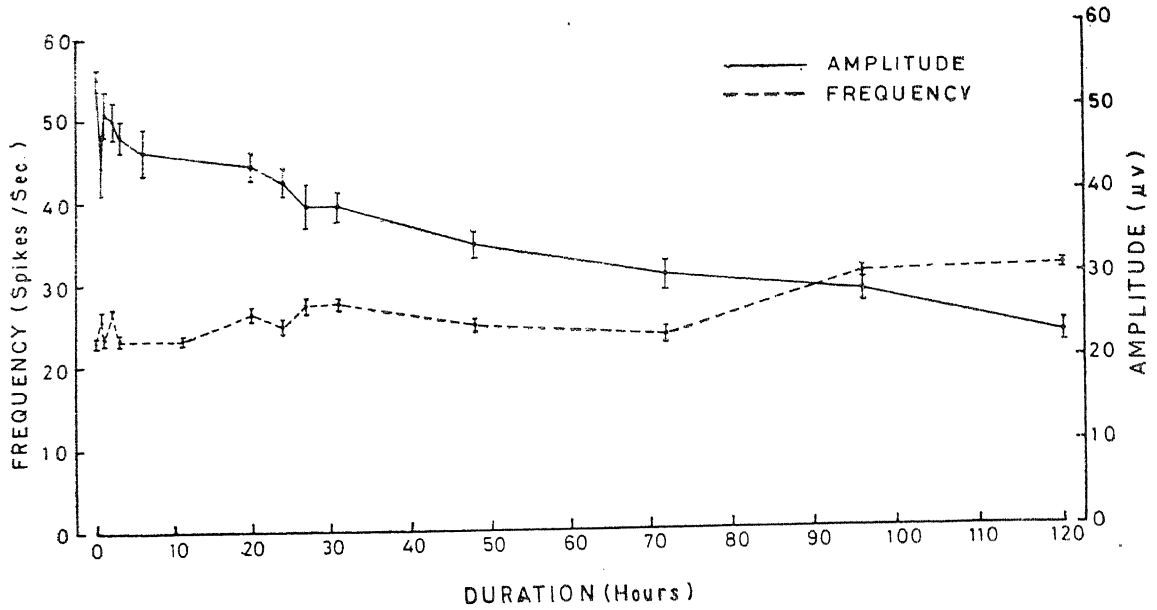

Fig. 1. Graph depicting the variations in frequency (---) and amplitude (—) of EEG spikes of adult mice at various *post infection* intervals. (Error bars indicate S. E. M.)

level. On the following intervals, there is an increase (1.6/sec.) at 5 % level of significance on the 24th h after HTO injection (20 μCi/g body weight). Whereas, on the 48th and 72nd h, no significant change in frequencies is noticed. On 96th and 120th h, frequency increased by 7.4 and 7.9/sec. respectively, as compared to the sham-irradiated or initial (0 h) frequency ($P < 0.001$ for both the intervals). When frequency on the 96th is compared with that on its precedent interval *i.e.* on the 72nd h, an increase by 6.9/sec. has been noticed ($p < 0.01$). Similarly amplitudes show continuous and gradual decline till the 120th hour post injection, with a maximum reduction about 1/3rd that of the initial.

Cahill *et al*[7]. showed that a continuous exposure of HTO and/or lead from conception of F_1 through F_2 generation in rats results in delayed CNS development revealing a low voltage and high-activity in their EEG and a decrease in relative brain weight of neonates. F_1 offsprings of female rats exposed to tritiated water from adolesence until and throughout pregnancy had abnormal body weight and cerebral weights. Besides cerebral DNA, protein and protein/DNA showed significant decline[8]. Soma and synaptic functions can be directly affected by changes in metabolites. These changes in EEG of exposed adult mice observed in the present investigation may be, therefore, attributed to alterations in functions at cortical level in neuronal and synaptic activities[9].

Our previous studies of tritium toxicity on adult gerbil brain has revealed a gradual derangement of neural architecture of cerebral cortex[10]. Hence such HTO-induced changes may be possibly a direct consequence of these structural deformities which warrants a careful evaluation.

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Effects of an organochlorine insecticide, thiodan on the oxygen consumption of the marine crab, *Scylla serrata* (Forskal) in relation to the changes in salinity

(insecticide/marine crab/oxygen consumption/salinity variations)

R. Biswas and R. V. Shrotri

Department of Zoology, Institute of Science, Bombay–400 032

ABSTRACT Juvenile crabs exposed chronically to three sublethal concentrations of thiodan showed a decrease in the oxygen consumption. Toxicity of thiodan as indicated by oxygen consumption was found to be salinity-dependent, when crabs were acclimated to selected salinities.

Pollution of water bodies by pesticides is an excellent example of how human intervention in an ecological system has been for benevolent purposes but have had a malignant effect. Marine pollution is currently a major problem because in certain respects it exclusively affects the fishing industry. Marine pollution due to pesticides needs considerable attention because of its harmful effects on marine organisms with special reference to edible products. They are vulnerable to certain pollutants like insecticides because of the tendency of these compounds to diffuse in the drainage systems and to concentrate in the coastal waters and estuaries. The account is part of a continuing programme to evaluate the impact of pesticides on estuarine fauna. The present work deals with the effect of thiodan, an organochlorine insecticide, on oxygan consumption of the edible marine crab, *Scylla serrata.* Estuaries are dynamic ecosystems where the very survival of an organism depends on its capacity to adjust to the salinity changes. Such salinity changes are in turn expected to affect the susceptibility of the estuarine organisms to adverse effects of different pollutants. Hence, in the present study, changes in oxygen consumption due to the combined stress of salinity variations and thiodan intoxication have been evaluated which could thus be employed as an useful indicator of metabolic stress.

Juvenile *S. serrata* of the intermolt stage and of more or less uniform size (33–35 mm carapace length) were acclimated to the laboratory conditions in large glass aquaria for 7 days before being used for the tests. Filtered sea water, which was used, had a salinity of 30–31‰, temperature of about 29°C, pH 8 and dissolved oxygen 7.2 ml/l. During acclimation as well as during the entire experimental period, lasting for 30 days, the crabs were fed on marine bivalve flesh every alternate day. Acclimated crabs were then exposed to three sublethal concentrations of thiodan. They were 0.1 ppm, 0.2 ppm and 0.3 ppm which were 1/6 1/3 and 1/2, respectively, of its 96h LC_{50} value. A separate control tank was also maintained in which acetone was added in an amount equal to the one used for the highest concentration dilution of thiodan. A duplicate set of these experiments was simultaneously run for confirming the results.

The oxygen-consumption tests were carried out on the 7th, 15th and 30th day of exposure to the experimental insecticide. During the experiments black bottles (3 litre capacity) were used as respirometers. Crabs exposed to thiodan were removed from their respective test aquaria and were transferred into these respirometers which were then filled with appropriately dosed sea-water using inlet pipes. Bottles were made completely air-tight and sealed with wax. Side by side control experiments were set up similarly except that the respirometers contained unexposed crabs and sea water without any insecticide. Before the start of the experiment, the initial oxygen content of the sea-water used in the tests was determined. All the experimental and control bottles were left undisturbed for one hour. Thereafter the water from each respirometer was carefully siphoned out to estimate its oxygen content. The dissolved oxygen was measured by the Winkler's Iodometric method[1]. The oxygen consumption rates (ml O_2/h/g wet wt.) were calculated from the differences between control and experimental values. Five crabs from each of the test aquaria and the control were subjected to the same experimental procedure. At the end of the experiment, each crab was removed and weighed accurately.

Following the same procedure as above, oxygen consumption tests were also carried out on crabs preacclimated for 1 week to different salinities 8‰, 16‰, 22‰ and 30‰. Experimental crabs were exposed to one of the sub-lethal concentrations of thiodan *i.e.* 0.2 ppm for a period of 30 days to variations in salinity–8‰, 16‰, 22‰ and 30‰. Control experiments under salinity stress were simultaneously run.

From the data presented in Table 1 it can be seen that there is no significant change in the rate of oxygen consumption of the control crabs during the 30 day test period. But there is a gradual decrease in the rate of oxygen consumption in the treated crabs, and the oxygen consumed at the end of the treatment period of 30 days differs significantly from the control. Similar reduction in oxygen consumption has been reported in fish exposed to different pesticides[2-4]. This inhibition of oxygen consumption rates in *S. serrata* may be related to the uptake and accumulation of thiodan in tissues of the exposed crabs. Such a relationship has been reported by Rao *et al.*[5] in fish *Macrognathus aculeatum* which showed reduced oxygen consumption when exposed to lethal and sublethal concentration of endosulfan. This was accompanied by an accumulation of endosulfan in tissues like brain, gills, gut, liver and kidney.

TABLE 1

Rate of oxygen consumption in *S. serrata* in ml of O_2/g wet wt/h

Exposure in days	control	concentration of thiodan in ppm/litre		
		0.1	0.2	0.3
Initial	0.1269 ±0.032	0.1266 ±0.031	0.1216 ±0.029	0.1271 ±0.036
7	0.1265 ±0.033	0.1154 ±0.066	0.1126 ±0 091	0.1036 ±0 0824
15	0.1285 ±0 042	0.1075 ±0 056	0.0921 ±0.063	0 0891* ±0.049
30	0.1302 ±0.068	0.0814** ±0 046	0.0802** ±0.035	0.0708** ±0.022

± = Standard Deviation
* = Significantly different from control ($P<0.05$)
** = Significantly different from control ($P<0.01$)
Sea water characteristics, Temp. 29°C; pH 7.9; Dissolved oxygen 7.2 ml/litre : Salinity – 30‰

Table 2 shows that oxygen consumption rates are inversely related to salinity; as can be seen from the highest rate of oxygen consumption in crabs exposed to 8‰ salinity after 7 days. Higher salinities of 30‰ and 22‰ have lesser effects on oxygen-consumption which may be due to the fact that the crabs used for the experiments were captured in waters with a salini y of approximately 30–35‰. Resulting minimal osmotic stress may then account for the ability of these crabs to tolerate the thiodan stress. However, radical changes in salinity like 16‰ and 8‰ produced an osmotic stress which in

TABLE 2

Rate of oxygen consumption in ml/g wet wt./h in *S. serrata* exposed to one sublethal concentration of thiodan (0.2 ppm/litre) under variations of salinity (S‰)

Exposure in days	S-30‰		S-22‰		S-16‰		S-8‰	
	Control	Treated	Control	treated	Control	Treated	Control	Treated
Initial	0.1269 ±0.032	0.1216 ±0.029	0.1273 ±0.037	0.1254 ±0.039	0.1260 ±0.028	0.1234 ±0.051	0.1253 ±0.043	0.1263 ±0.048
7	0.1265 ±0.033	0.1126 ±0.091	0.1259 ±0.041	0.1271 ±0.046	0.1277 ±0.054	0.1299 ±0.045	0.1294 ±0.029	0.1491* ±0.021
15	0.1285 ±0.042	0.0921 ±0.063	0.1264 ±0.061	0.1058 ±0.071	0.1283 ±0.043	0.0957 ±0.037	0.1297 ±0.056	X
30	0.1302 ±0.068	0.0802** ±0.035	0.1298 ±0.059	0.0793** ±0.048	0.1308 ±0.036	X	0.1313 ±0.046	X

± = Standard Deviation

* = Significantly different from control ($P<0.05$)

** = Significantly different from control ($P<0.01$)

X = Did not survive

turn increased their susceptibility to thiodan causing early mortalities. Exposure to low salinities even in normal crabs is accompanied by a reduction in the blood osmotic and ionic concentrations followed by active ion uptake leading to osmoregulatory adjustments[6]. Kirschner[7] stated that the amount of energy that is required to osmoregulate is expressed by appreciable changes in resting oxygen consumption. Dehnel and Carefoot[8] observed that blood hypertonicity in a dilute environment involves the participation of energy consuming processes which can account for the observed higher oxygen consumption rates at low salinities due to higher energy requirements.

Environmental variables like salinity greatly influence the toxicity of insecticides and an evaluation of the biological response in terms of oxygen consumption has shown that salinity stress coupled with the effect of thiodan increases the metabolic rate. It in turn makes them consume more oxygen at lower salinities with a gradual decrease in the rate on prolonged exposure and eventual death.

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Histopathological changes induced by Epibloc (U_{5897}) in the liver and kidney of black rats *(Rattus rattus)*

(Epibloc/*Rattus rattus*/liver/kidney)

SEEMA RANI AND S. S. LAL

Department of Zoology, Institute of Advanced Studies, Meerut University, Meerut-250 005

ABSTRACT Single dose of Epibloc at two dose levels (135 mg/kg and 400 mg/kg) by gavage produced several pathological changes in liver and kidney of black rats (*Rattus rattus*). Histology of liver showed dilatation of central vein, fibrosis and inflammatory reaction at periportal zone. cytoplasmolysis, degeneration of hepatocytes alongwith karyolysis, karyorrhexis and karyopyknosis, and fatty changes after 400 mg/kg of Epibloc treatment. Histological changes seen in kidney were marked degree of cloudy swelling in tubular epithelium, tubular cast in few of nephric tubules, loss of brush border of proximal convoluted tubules, congestion in glomeruli and in perinephric area, increase in subcapsular spaces and thickening of basement membrane. karyolysis, karyorrhexis and pyknosis in nuclei of tubular epithelium (400 mg/kg).

Epibloc (3-chloro-1, 2 propanediol) acts as good sterilant as well as good toxicant and hence is widely used for the control of rat population in the world. The compounds α-chlorohydrins were found to have antifertility activity in male rat. A series of structurally related compounds of which U-5897 (3-chloro-1, 2 propanediol) is a member possess antifertility efficacy in rat[1]. It was observed that male rats receiving daily injections of α-chlorohydrin (U-5897) at a dose of 7 to 8 mg/kg became infertile[2,3]. Many attempts have been made to evaluate the antifertility effect of Epibloc but no work is available regarding toxic effect of Epibloc on liver and kidney. The present investigation includes the effects of a single dose of Epibloc on histopathological changes in the liver and kidney of black rats (*Rattus rattus*) which occurs widely as a rodent pest in this region.

Epibloc concentrate (liquid with 20 per cent W/W active ingredient) was diluted in distilled water at two dose levels (135 mg/kg and 400 mg/kg). Diluted Epibloc (in distilled water) was administered by gavage in terms of mg of active ingredient of Epibloc/kg of body weight of rat. All the animals were autopsied after 7 days of Epibloc administration.

The liver and kidney of all the animals (treated and control) were removed and stripped of excess tissues and fixed in Bouin's fluid. The tissue was dehydrated in a series of graded ethanols and embedded in paraffin. Sections (6 μm) were cut and stained with haematoxylin and eosin.

Liver : 135 mg/kg - There is mild dilatation of central vein. Degenerative changes are mainly seen in peripheral zone of the liver lobule. Hepatocytes showed cloudy degeneration alongwith nuclear alterations, karyopyknosis, karyolysis and karyorrhexis. Portal area shows mild fibrosis and presence of few inflammatory cells (compare Figs. 1 and 2).

400 mg/kg - There is a moderate degree of dilata-

tion of central vein. Hepatocytes exhibited uniform fatty changes throughout the liver lobules showing singeltlike-appearance. Periportal fibrosis and presence of inflammatory cells is well marked. Cytoplasmolysis is also noticed. Karyopyknosis, karyolysis and karyorrhexis are well marked with degeneration in hepatocytes (Fig. 3).

Kidney : 135 mg/kg – Glomeruli show proliferation of tuft and congestion. Tubules show cloudy swellings alongwith cytoplasmic and nuclear changes (karyopyknosis, karyolysis and karyorrhexis). Interstitial tissue shows few dilated blood vessels. Brush border of proximal convoluted tubules becomes irregular. Periportal haemorrhage is frequently observed (compare Figs. 4 and 5).

400 mg/kg – Blood vessels in interstitial tissue are dilated. Marked degree of cloudy swelling in tubular epithelium is evident. Some of the nephric tubules exhibit degeneration of epithelium and tubular casts. Brush borders are absent in proximal convoluted tubules. Congestion in glomeruli and perinephric areas is noticed. Glomeruli become quite indistinct due to patches of protoplasmic material and large sized vacuoles. Renal capsules reveal increase in subcapsular spaces alongwith thickened basement membrane. Nephric tubules exhibit karyolysis, karyorrhexis and pyknosis in nuclei of tubular epithelium (Figs. 6 and 7).

Present investigations reveal extensive pathological fibrosis at portal area induced by Epibloc. Similar results have been reported after cadmium intoxication[4]. A remarkable pathological change in liver of Epibloc (400 mg/kg) treated rats are the fatty changes. Fatty infiltration in liver of rodents has been reported by several workers[5-8]. In fatty change the liver cell is considered to be firstly damaged resulting in deranged metabolism and followed by the appearance of fat in the cells. There may be two possible sources of fat in hepatocytes (i) the extracellular lipid metabolic pool, or (ii) the intracellular fat which is often present in the hepatocytes in sufficient quantity. Too much fat may be transferred to the cell or the fat already in the cell may be made visible by unmasking. It may now be suggested that fat metabolism of hepatocytes might be interfered by Epibloc administration which results in fat accumulation in hepatocytes.

The nephric tubules show mild reaction of chronic inflammation suggesting tubular degeneration after Epibloc treatment (400 mg/kg). The tubules show marked degree of cloudy swellings in kidney of 135 mg/kg and 400 mg/kg treated rats. Infiltration of inflammatory cells in cortical and medullary regions could have brought about tissue changes. Focal inflammatory reaction by mononuclear cells was noticed in the interstitium of renal cortex in rats[9]. On the basis of present studies it can be suggested that Epibloc primarily acts upon nephric tubules causing degeneration and cloudy swellings. Glomeruli were affected only in the later stages.

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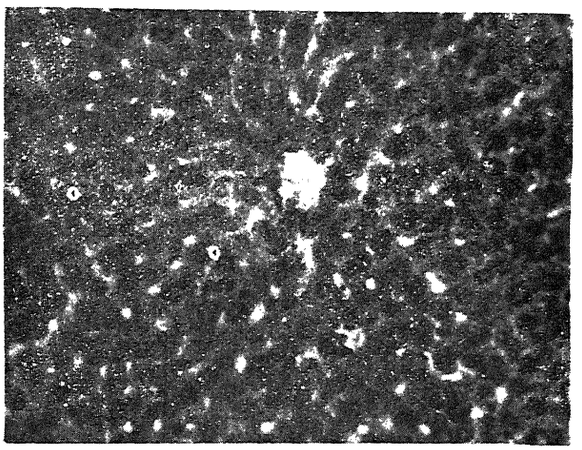

Fig. 1. T. S. liver from control rat showing normal histologic make up of central vein and hepatocytes × 400.

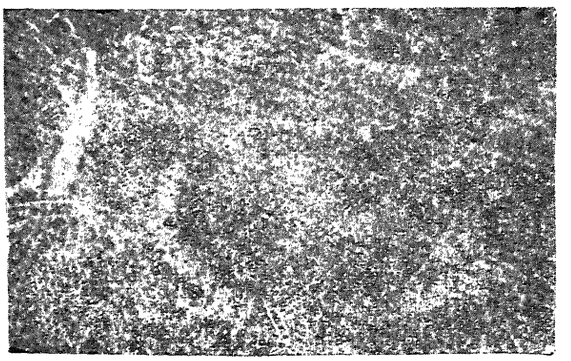

Fig. 2. T. S. liver from rat treated with Epibloc-135 mg/kg showing fibrosis and mild inflammatory reaction × 100.

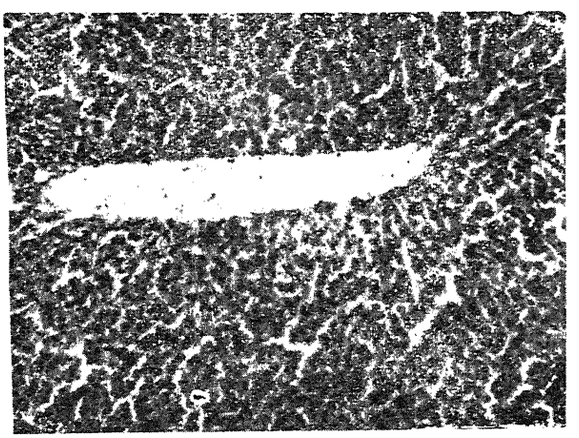

Fig. 3. T. S. liver from rat treated with Epibloc-400 mg/kg showing marked degree of fatty changes and degeneration in hepatocytes × 100.

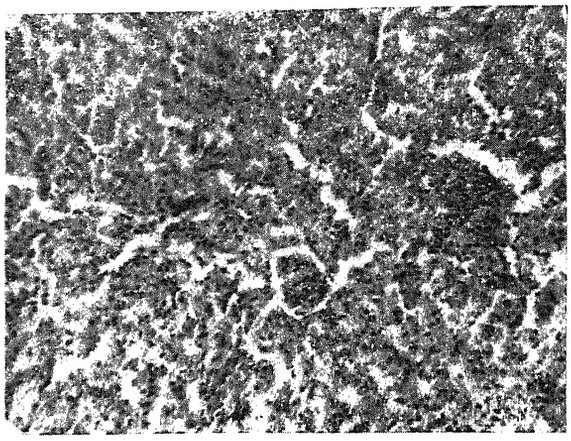

Fig. 4. T. S. Kidnev from normal rat showing normal histologic make up of cortex region × 150.

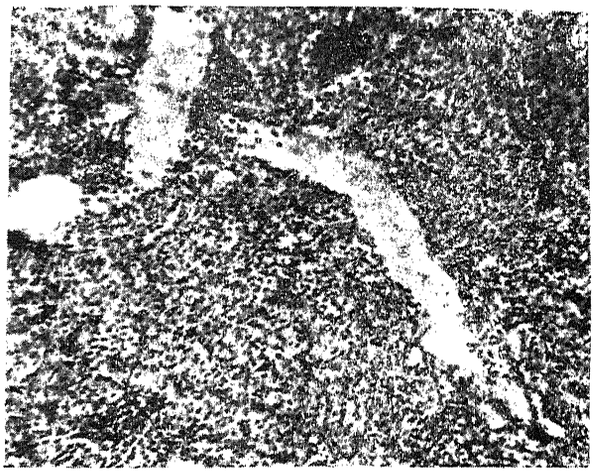

Fig. 6. T. S. Kidney from rat treated with Epibloc-400 mg/kg showing dilatation and congestion of blood vessel alongwith few inflammatory cells around it × 150.

Fig 5. T. S. Kidney from rat treated with Epibloc-135 mg/kg showing marked degree of cloudy swelling, cytoplasmic and nuclear changes - Karyorrhexis, karyolysis and pyknosis and infiltration of inflammatory cells × 150.

Fig. 7. T. S. Kidney from rat treated with Epibloc-400 mg/kg showing proliferation of glomeruli, degenerative and nuclear changes - karyopyknosis, karyolysis, karyorrhexis and infiltration of inflammatory cells × 150.

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   Osgood, C. F. (1977) in *Number Theory and Algebra*, ed. Zassenhaus, H., Academic Press, New York, p. 321. (For edited Books)

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(e) Key words : A maximum of 5 key words should be supplied. Each of these words will be separated by a slash (/) and printed just below the title of the research paper, e. g. (steroid receptors/protein-DNA interactions/gene regulation).

(f) Acknowledgements, if any, should appear at the end of the letter, but just before the references.

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